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(54) Title: FETAL MEMBRANE TUBES FOR NERVE AND VESSEL GRAFTS

(57) Abstract

Disclosed are cylinders having a wall formed of at least one layer of sterilized cross-linked Types I, II, III collagen or combinations thereof from placenta for nerve and blood vessel grafts, methods of manufacture and use. The nerve grafts promote axon regeneration therethrough. The nerve and blood vessel grafts are non-immunogenic, can be constructed into tubes of various lengths and diameters, are easily accessible and are patent or open in use.

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FETAL MEMBRANE TUBES FOR NERVE AND VESSEL GRAFTS

Field of the Invention

The present invention is directed to fetal membrane tubes for nerve and vessel grafts.

Background of the Invention

Fetal membranes, amnion and chorion, have been selected as our starting material for several reasons. The fetal membranes preferably are human although animal membranes can be used. (1) In one aspect of the invention, we have demonstrated a low immunogenicity of the human amniotic and chorionic conduits in rats which was evidenced when different antigens in their membranes, mainly collagen types I, II, and III fibronectin, and laminin were tested by dot blot and ELISA techniques. (2) Human placentas are available in relatively unlimited quantities and at low cost. (3) For almost 90 years amnion and chorion have been used for a wide variety of medical and surgical indications. (4) They have been shown to be capable of neovascularization. (5) They have also been placed in subcutaneous pockets in human without evidence of acute rejection for as long as 7 weeks. The fetal membranes are of a complex biochemical structure with unique physical characteristics. They can be modified physically by the processing technique later described herein into conduits which are semi-rigid, resilient, and of variable length, diameter and thickness. The biochemical components of the amnion and chorion membranes are mainly collagen types I, II, and III, laminin, fibronectin and other glycoproteins. Laminin has been shown to promote axon extension by

interacting with axonal glycoproteins that are members of the integrin family of receptors. The immunocytochemical studi s we conducted led us to modify our processing technique of the amnion and chorion membranes in order to preserve laminin as a significant component of these conduits, as later described herein.

While human amnion and chorion membranes are preferred, these membranes from other animals, such as bovine membranes, are another aspect of the present invention.

Clinically, it is accepted that the use of a nerve autograft is the method employed to reconstruct a nerve gap. The disadvantages of nerve autografts are shown to be many, i.e. an additional surgical procedure is required, scarring with anesthesia or hyperesthesia at the donor site may be a problem, and there frequently are dimensional limitations of the donor grafts. Although nerve allografts have overcome several disadvantages of the autografts, rejection of the graft remains a major problem and limits its clinical use despite the use of immunosuppressive agents.

The current interest and future directions in nerve research focus on the development of an ideal nerve conduit for clinical use. Several investigators have studied the use of different materials including silicone rubber, PTFE, polyorthoester, polyglactin, mesothelial tubes, muscle basal lamina, and vein grafts as nerve conduits. Each of these materials mentioned has shortcomings and none have proven to provide the ideal environment for the regenerating nerve. An ideal nerve conduit should be readily available, of low cost, easily manufactured, of different sizes, non-immunogenic, microporous, noncollapsible, biodegradable, and of biochemical components that provide a favorable environment for the regenerating axons. As later described herein, we have developed a nerv conduit using human amnion membrane which possess s many of the characteristics of the proposed ideal nerve conduit.

A number of investigators are currently exploring the use of collagen for nerve conduits. By necessity, this collagen is either allogenic or xenogeneic in origin and, thus, could stimulate an immune response that may inhibit nerve regeneration. Immunological testing of the amniotic and chorionic nerve conduits of the present invention showed a minimal, nonsignificant immune response, thus, avoiding some of the inhibiting factors that could influence the regenerating nerve. Although amniotic and chorionic conduits showed signs of biodegradability and structural reorganization grossly and by electron microscopic examination, local inflammatory cells infiltration was slight, being limited to the area surrounding the conduit wall, and it did not produce changes similar to those of chronic nerve compression. This latter development is a major problem encountered with the use of silicone rubber conduits for nerve regeneration.

Functional and morphological assessment of nerve regeneration using amniotic and chorionic conduits proved its superiority to nerve autografts and silicone tubes.

Also, there is a need for blood vessels which can be connected to their proximal and distal ends when blood vessels are severed such as when thrombosis occurs and blood vessel segments are removed. In bypass operations, veins stripping in the legs for these purposes can be avoided and similarly in subsequent bypass operations as the patient does not have adequate vein to take the place of the thrombosed arteries which are removed. Also, vein graft wall collapse and obliteration of its lumen added to the donor's site morbidity are significant problems which limit their use. Vascular conduits commonly used are vein grafts harvested from the patient's legs or arms. The donor site morbidity and the unpredictable patency of vein grafts for coronary bypass and peripheral vascular interposition bypass grafts are well known. Several vascular conduits have been tried and in common use including preserved bovine vein grafts, dacron prosthetic grafts, teflon, and umbilical artery grafts. The

high incidence of the thrombosis and infection of these grafts is also a problem. To construct an ideal vascular graft, the graft should be non-thrombogenic, easily accessible, of variable diameters and lengths, extensible, and flexible.

Amniotic and chorionic collagen tubes or conduits according to the present invention are non-immunogenic and can be constructed into tubes of variable lengths and diameters readily and easily. The amniotic and chorionic tubes of the present invention are well suited for use as nerve grafts or as bypass conduits for coronary bypass or vascular bypass surgery.

Summary of the Invention

The present invention is directed to providing nerve and vessel tubes or conduits for grafting to bridge gaps in nerves and vessels. The physical characteristics of the Types I, II, III collagen derived from the amnion and chorion placentas in membrane form have been modified and the tubes constructed so that they are maintained patent and flexible so blood flows through their lumen and their walls can withstand the interstitial pressure, and in which the fetal side or shiny side of the membrane is the inward side, which in the case of nerve grafts promotes axon growth.

In one aspect of the invention, to provide such a graft, amnion and chorion is obtained from fresh placentas, preferably human, the amnion and chorion layers are separated from the placenta and each other, cellular monolayer material overlying the basal lamina on the fetal side of the membrane is removed, such as by exposure to trypsin or pepsin, the amnion and chorion is rinsed repeatedly with phosphate buffer solution or distilled water until clean, the amnion or chorion is then cross-linked either by exposure to gamma radiation or chemical cross-linking such as with glutaraldehyde, which sterilizes the tissue, provides protection against viral dis as transmission, strengthens and permits remodeling the material from sheet to conduit form. The amnion and chorion she ts are then wrapped in layers so that the fetal surface,

which is shiny, is directed toward the inner surface of the finish d tube. The number of wraps will depend upon the length and diameter of the tube. The tubes are then dried and placed in bottles which are sealed, labeled and, if desired, exposed to 2.5 M rads of gamma radiation to again sterilize and further cross-link the conduit collagen. If desired, the layers can be glued together by a suitable glue, such as a fibrin glue, to prevent delaminating, particularly in larger conduits, such as used for vascular grafts.

The tubes or conduits can then be stored, for example at -20°C, until used.

For nerve grafts, nerve promoting factors can be used within the amnion and chorion tubes at the time of implantation, for example particles of basal lamina, fibronectin, collagen extract, nerve growth factors and other related growth factors.

Accordingly, it is an object of the present invention to provide a tubular graft for joining proximal and distal stumps of a severed peripheral nerve or proximal and distal ends of a vessel which avoids the foregoing disadvantages of the prior art and has the advantages mentioned above.

It is a further object of the present invention to provide a graft for joining proximal and distal stumps of a severed peripheral nerve or proximal and distal ends of a vessel which comprises a cylindrical wall formed by layers of sheets of sterilized collagen, Types I, II, III, from human placenta from which its cellular material is substantially removed, which has its fetal side directed inwardly and its collagen cross-linked by irradiation or chemically, the cylinder having sufficient layers to maintain the cylinder patent or open, the cylinder having a length greater than the distance between the proximal and distal stumps or ends, and having a diameter at least equal to connecting tissues of the distal stumps or ends.

A further object of the present invention is to provide such a graft in which the lay rs of amnion and/or chorion membranes are glued together effective to prevent delamination of the layers.

It is still a further object of the present invention to provide such a cylindrical graft in which its wall permits flow of interstitial fluid through it to provide early nourishment for the graft, and in the case of nerve grafts, to provide nourishment for growth of Schwann cells.

It is a still further object of the present invention to provide such a cylindrical graft as a research model for inclusion of material for medication study of nerve regeneration in the field of neurology.

It is a further object of the present invention to provide a nerve graft comprised of an amniotic and/or chorionic tube containing related growth factors.

It is a further object of the present invention to provide a nerve graft comprised of an amniotic and/or chorionic tube containing basal lamina particles.

It is a further object of the present invention to provide such a graft which can be made in relatively long lengths, which will not kink, will retain its shape and in which the passageway remains patent or open.

Other and further objects, features and advantages of the invention appear throughout.

Description of Preferred Embodiments

A. Amnion Harvesting and Preparation

In this aspect of the invention, amnion is obtained from fresh human placentas. The placentas are from hospital labor and delivery within 24 hours of parturition. Placentas obtained only from mothers who have been screened for AIDS and hepatitis virus and who are not members of the high risk groups such as IV drug abusers are used. Care is taken to avoid skin contact with blood and tissu and to minimize contamination of the work areas with these materials.

The amnion layer is separated from the placenta, such as by finger dissection. The largest possible pieces of amnion which are of uniform thickness are selected from all the amnion harvested. The selected pieces of membrane are thoroughly washed, preferably with phosphate buffered saline, or distilled water to remove all the blood and debris. Then the membranes are further washed until they are white and transparent.

The cellular monolayer overlying the basal lamina on the fetal side of the membrane is removed, such as by exposure to trypsin. Membranes are immersed for two hours at room temperature in 1:1 solution of distilled water and trypsin. The trypsin used preferably is from the procine pancreas at a 25% concentration without calcium or magnesium. Following treatment with trypsin, the amnion is rinsed repeatedly, preferably with phosphate buffered saline, or with distilled water until clean, white membranes with no trace of pink trypsin are obtained.

Rinsed amnion sheets are bottled in distilled water and exposed to 500,000 rads of gamma radiation. Irradiating the amnion cross-links the collagen, sterilizes the tissue, provides animal protection against viral disease transmission and subsequent remodeling of the material from sheet to conduit or tubular form. The bottles are then stored in a freezer at -80°C. If desired, the amnion sheets may be cross-linked chemically such as with glutaraldehyde.

B. Conduit Manufacturing

Amnion is removed from frozen storage and then thawed for 3 to 4 hours at room temperature. The amnion sheets are examined under the operating microscope for defects and determination of the fetal or shiny side of the membrane. By using a rolling machine, sheets of the amnion wide enough for a desired conduit length and diameter are carefully collected. The amnion sheets are oriented so that the fetal surface, which is shiny, would be directed towards the inner surface of the finished tube.

Preferably, wrapping of the amnion she ts in layers is effected by using a highly polished stainless st el stent of the appropriate diameter, although the amnion sheets can be wrapped in any desired manner.

The number of wraps or layers of the amnion sheets necessary to maintain the amnion cylinder's shape, avoid kinking and to keep it patent or open depend upon the length and diameter of the tube or cylinder. For example, conduits of 1.6-1.8 inches in diameter require approximately 10 wraps of amnion and 15 wraps are satisfactory for a diameter of 2.5. The conduits can be of any desired length. The tubes which are on the stents are then dried in 40-60°C oven for about 30 minutes. Drying allows the tubes to be removed from the stents easily. If desired, to prevent delamination, a suitable adhesive or glue, such as fibrin glue, can be used to glue the layers or wraps of amnion sheets together. The tubes are then placed in bottles which are sealed, labeled, and exposed to 2,000,000 rads of gamma radiation to again sterilize and further cross-link the conduits collagen. After that, the conduits are stored in -20°C until used.

Example 1

In this example, the basement membrane integrity and lamina content of human amnion before, and after, the construction of the conduits were evaluated by immunocytochemical methods. Human amniotic conduits according to the invention have low immunogenicity which was evidenced when different antigens in the amnion membrane, mainly collagen type 1, fibronectin, and laminin were tested by dot blot and ELISA techniques. For almost 90 years amnion has been used for a wide variety of medical and surgical indications and has been shown to be capable of neovascularization. It has also been placed in subcutaneous pockets in human without evidence of acute rejection for as long as 7 weeks.

The f tal membranes are of a complex biochemical structure with unique physical characteristics. They ar

modified physically in the present invention by the process technique described herein into conduits which are semi-rigid, resilient, and of variable length, diameter and thickness. The biochemical components of the amnion membrane are mainly collagen type I and type III, laminin, fibronectin and other glycoproteins. Laminin has been shown to promote axon extension by interacting with axonal glycoproteins that are members of the integrin family of receptors. The methods of processing the amnion membrane of the present invention preserve laminin as a significant component of these components. The present method of using gamma irradiation has several advantages. It has been shown that all bacteria, fungi, and viruses (including AIDS) are destroyed at .20 M rads. Preferably, a dose of 2.5 M rads is used in order to ensure complete sterility of the final material. This dose also increases amniotic collagen cross-linking which strengthens and thus enables the manufacture of the amniotic conduits in a semi-rigid form while retaining some resiliency to maintain patency after implantation. As indicated previously, however, chemical cross-linking by known methods, such as exposure to glutaraldehyde, is effective and satisfactory.

By immunological testing, the amniotic nerve conduit showed a minimal, nonsignificant immune response, thus, avoiding some of the inhibitory factors that could influence the regenerating nerve. Although amniotic conduits showed signs of biodegradability and structural reorganization grossly and by electron microscopic examination, local inflammatory cells infiltration was slight, being limited to the area surrounding the conduit wall, and it did not produce changes of chronic nerve compression. This latter development is a major problem encountered with the use of silicone rubber conduits for nerve regeneration recently presented for clinical use.

Long-term studies of the amniotic conduits showed major structural changes as evidenced by Schwann-lik cells

infiltration through the layers of conduit and collagen reorganization, indicating a phenomenon of biodegradability and reorganization, a characteristic unique to the amniotic conduit.

As previously mentioned, the amniotic tube of the present invention can be used as a carrier for many materials that promote nerve regeneration, such as lamina, fibronectin, collagen extract, nerve growth factors, and other related factors. Collagen extract is collagen extracted from human fetal membranes such as described in U.S. Patent No. 5,002,071. For example, basal lamina can be used as freeze dried ground, polarized, or preserved in any preservative solution within the amnion tube at the time of implantation.

Example 2

In this example, nerve regeneration through a conduit according to the present invention was compared morphologically and functionally with autographs and other types of nerve tubes in an experimental animal model.

Eleven cats were divided into five groups to assess nerve regeneration through a 4 cm gap of the tibial nerve.

Group 1 (3 animals) amnion tube.

Group 2 (3 animals) amnion tube and basal lamina as a neurotrophic factor extracted form muscle.

Group 3 (3 animals) nerve autograft.

Group 4 (1 animal) sham operation as a control.

Group 5 (1 animal) no repair.

The animals were followed for six months when they were harvested, and the nerve segments were studied morphologically and histologically.

The cats were anesthetized using Demerol and phenobarbital intramuscular and a mixture of Halothane and No_2 by inhalation. The tibial nerve was exposed, and a 4 cm s gment was excised and repaired as following:

In group 1 (amnion) an amnion tube was sutured to the distal and proximal stumps ensuring a gap of 4 cm. Two stitches were placed 180° apart n each side and w nt through

the whol thickness of the tube containing only the epineurium of the nerve stump.

In group 2 (amnion tube - basal lamina) - same as above, a 4 cm segment of the nerve was excised, and the gap was bridged by an amniotic tube filled with basal lamina.

In group 3 (nerve autograft) a 4 cm segment was excised, then reattached using epineural technique.

In group 4 (sham) the tibial nerve was explored and left undisturbed.

In group 5 (no repair) a 4 cm segment of the tibial nerve was excised, and no repair was ensured by suturing the nerve stumps to the underlying muscle.

The animals were followed for six months, and no functional improvement was noticed in any of the experimental groups excluding the sham.

At six months the cats were sacrificed and morphological and histological studies were performed. The results recommend amnion basal lamina as a strong candidate for nerve regeneration and show that the addition of muscle basal lamina to the amniotic collagen tube enhances nerve regeneration.

In axonal diameter histograms the amnion basal lamina group showed a distribution comparable to the sham with even larger axons and a considerable percentage of the axons (9%) fell in the range of from 1.5 to 2.25 microns. In the case of nerve autograft, the histogram was comparable with the sham but with more percentage of axons (37%) falling in the range from 0.5 to 0.75 microns. In the amnion tube group the axonal diameters ranged between 0.1 to 1 micron with most of the axons (58%) falling in the range 0.25 to 0.5 microns. The axonal diameter histograms showed that basal lamina helped in rendering larger axons and provided to be the closest to the normal.

The conclusions from this example are that human amniotic conduits are strong substitutes for nerve grafting and muscle basal lamina proved to be a good neurotrophic

material when added to the amniotic collagen tube. Amnion conduits filled with basal lamina are superior to nerve autografts. Human amniotic collagen tubes are good conduits for nerve regeneration and can be used to house any neurotrophic material.

Example 3

In this example, human chorion was substituted for human amnion. The chorion membrane was separated from the amnion membrane and was then harvested and treated the same as in paragraph A, and conduits formed the same as in paragraph B has properties similar to those set forth in Examples 1 and 2, and is satisfactory for both nerve growth and vascular tubes. The step of using trypsin or pepsin may be omitted if the chorion is separated from the amnion.

Example 4

In this example, collagen Types I, II, and II, and combinations thereof derived from bovine amniotic and chorionic membranes were substituted for human membranes and prepared and formed into conduits or tubes as described above and provide similar and satisfactory results for both nerve growth and vascular tubes as set forth above for the human membranes.

The methods of the invention comprise joining the proximal and distal stumps or ends of a severed nerve or proximal and distal ends of a vessel with a tube comprised of at least one layer of sterilized cross-linked membrane free of cellular or epithelial material comprising Types I, II, III collagen or mixtures thereof, laminin, fibronectin and other glycoproteins, having its fetal side directed inwardly. The membrane is sterilized and cross-linked by irradiation or chemically and has sufficient layers to maintain them patent in place. The tube has a length at least equal to the distance between the proximal and distal stumps or ends, and has a diameter at least equal and preferably slightly larger than connective tissue of the proximal and distal stumps or ends. For nerve grafts the tube can contain nerve growth

factors, such as basal lamina, fibronectin or collagen extract or combinations of them, as previously mentioned. The tube is sutured or otherwise secured to the connective tissue adjacent the nerve stumps or vessel ends.

The grafts are storable for extended periods of time providing a ready available source of graft material. The grafts can be formed of any required diameter and length, and the layers of the graft can be glued together to prevent delamination of the layers in use.

Accordingly, grafts for bridging a gap betwe n proximal and distal ends of a severed nerve or of a vessel comprising a cylinder having a wall formed of at least one layer of sterilized cross-linked membrane derived from Types I, II, III collagen or combinations thereof from placenta are suitable and satisfactory.

Accordingly, the present invention is well suited and adapted to attain the objects and ends and has the features and advantages mentioned as well as others inherent therein.

While presently preferred embodiments of the invention have been given for the purposes of disclosures, changes and modifications can be made within the spirit of the invention as defined by the scope of the appended claims.

What is claimed is:

Claims

1. A graft for bridging a gap between proximal and distal ends of a severed nerve and proximal and distal ends of a vessel comprising,

a cylinder having a length at least equal to the distance between the proximal and distal ends of the severed nerve or vessel and a diameter at least equal to the diameters of the proximal and the distal ends of the severed nerve or vessel,

said cylinder having a wall formed of at least one layer of sterilized cross-linked membrane, said membrane comprising collagen selected from the group consisting of Type I, Type II, Type III, and mixtures thereof,

said collagen being derived from the group consisting of amnion of a placenta, chorion of a placenta, and combinations thereof.

2. The graft of Claim 1 wherein,

said wall comprising at least two layers of said membrane in sheet form glued together effective to prevent delamination of the layers in use.

3. The graft of Claim 1 where,

the graft permits flow of interstitial fluid through its cylindrical wall.

4. The graft of Claims 1, 2 or 3 where,

the graft is a nerve graft and contains at least one nerve growth factor.

5. The graft of Claims 1, 2 or 3 where,

the graft is a nerve graft and contains basal lamina, fibronectin, amniotic collag n extract or combinations ther of.

6. A method for restoring nerve function of a severed move comprising,

joining the proximal and distal ends of the severed nerve with the graft of Claims 1, 2 or 3.

7. A method for restoring nerve function of a severed nerve comprising,

joining the proximal and distal ends of th severed nerve with the graft of Claims 1, 2 or 3,

the nerve graft containing at least one nerve growth factor.

8. A method for restoring nerve function of a severed nerve comprising,

joining the proximal and distal ends of the severed nerve with the graft of Claims 1, 2 or 3 containing basal lamina.

9. A method of replacing a blood vessel comprising,

joining the proximal and distal ends of the blood vessel with the graft of Claims 1, 2 or 3.

10. The graft of Claims 1, 2, 3, 4, or 5 where,

the amnion or chorion membrane is from a human placenta.